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STUDIES IN MICROBIOLOGY VOLUME 2

Principles of Gene Manipulation

AN INTRODUCTION TO GENETIC ENGINEERING

R. W. OLD MA, PhD
Department of Biological Sciences,
University of Warwick,
Coventry

S. B. PRIMROSE BSc, PhD
Amersham International plc,
Amersham,
Buckinghamshire

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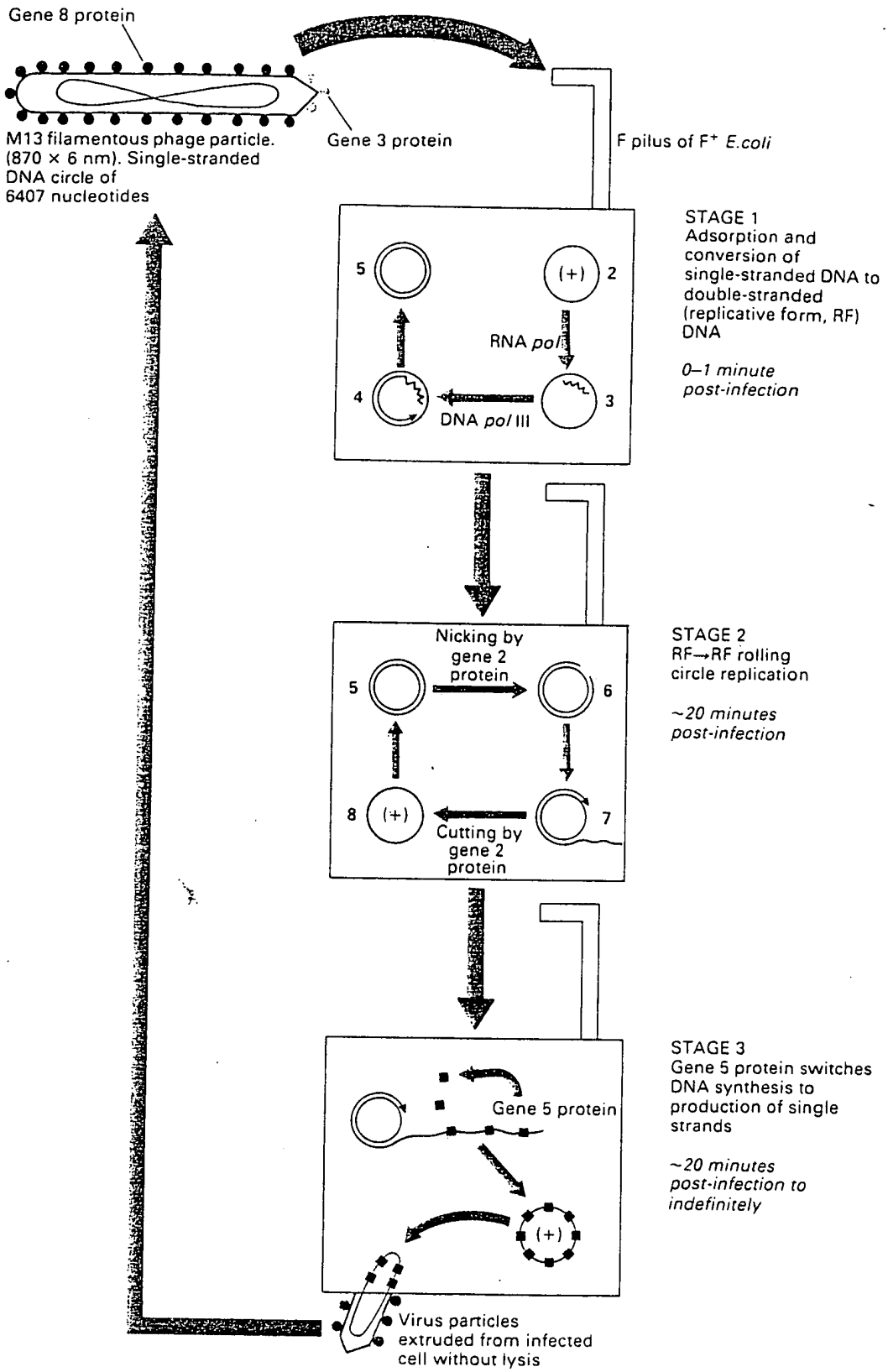


Fig. 4.10 Life cycle and DNA replication of phage M13.

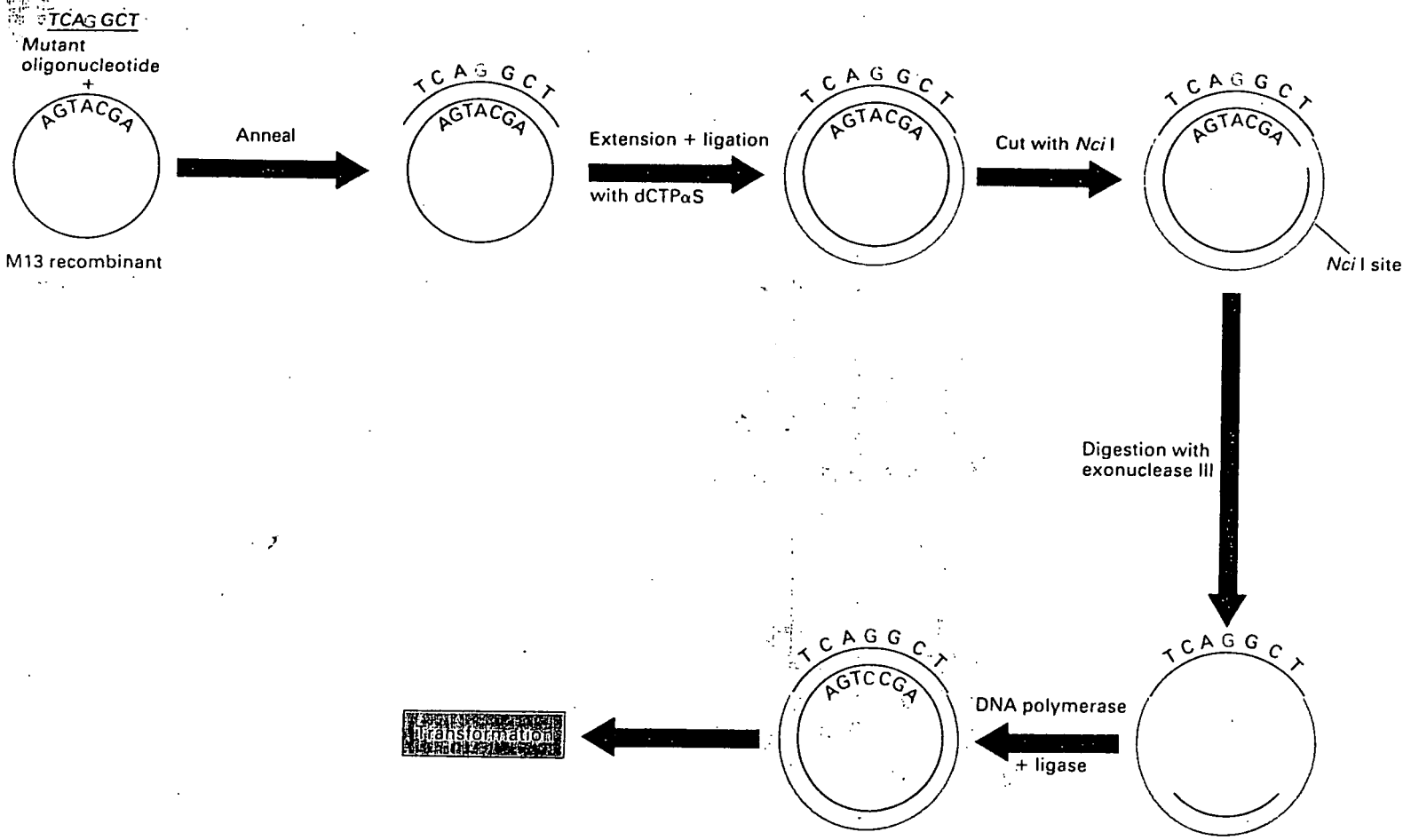


Fig. 5.7 *In-vitro* strand selection of mutants. The DNA-containing sulphur nucleotides is shown in colour.

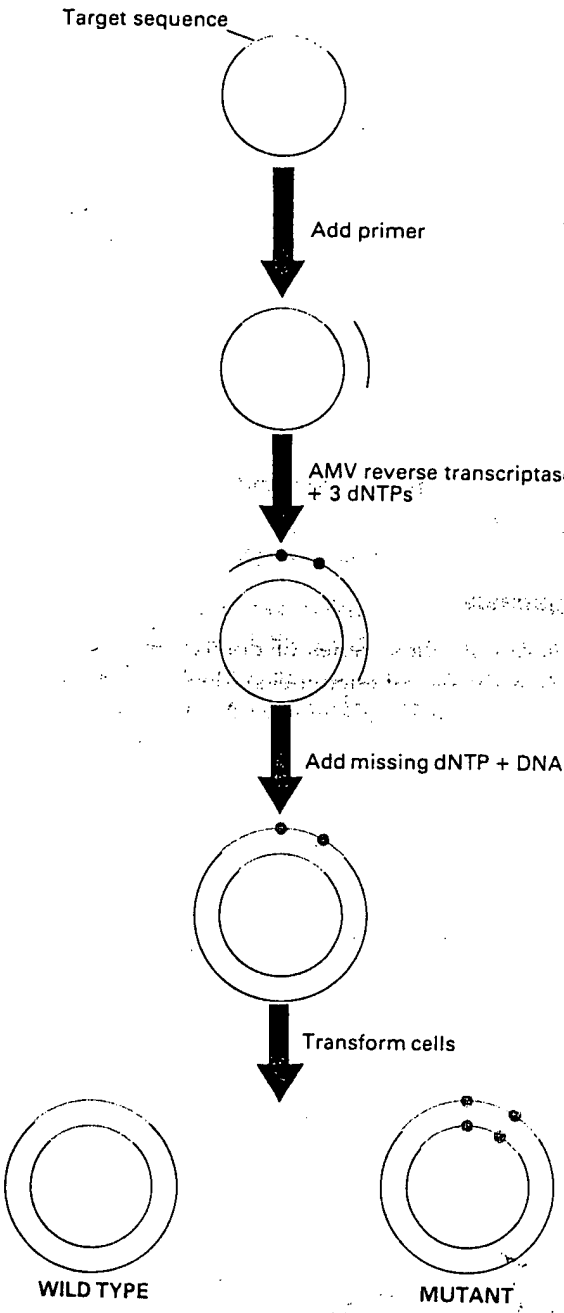


Fig. 5.9 Procedure for random mutagenesis of a target sequence. In the absence of one of the dNTPs the AMV reverse transciptase copies the template DNA slowly and inserts any of the dNTPs present for the missing dNTP. On adding back the missing dNTP, replication occurs at its usual high rate. Dots represent mutated bases.

could correct the *CYC-1* mutation, and revertants were obtained that had the expected base sequence. For this method to succeed it is necessary to have stable mutants and a positive selection method for revertants. High DNA concentrations (100 μg) also are required.

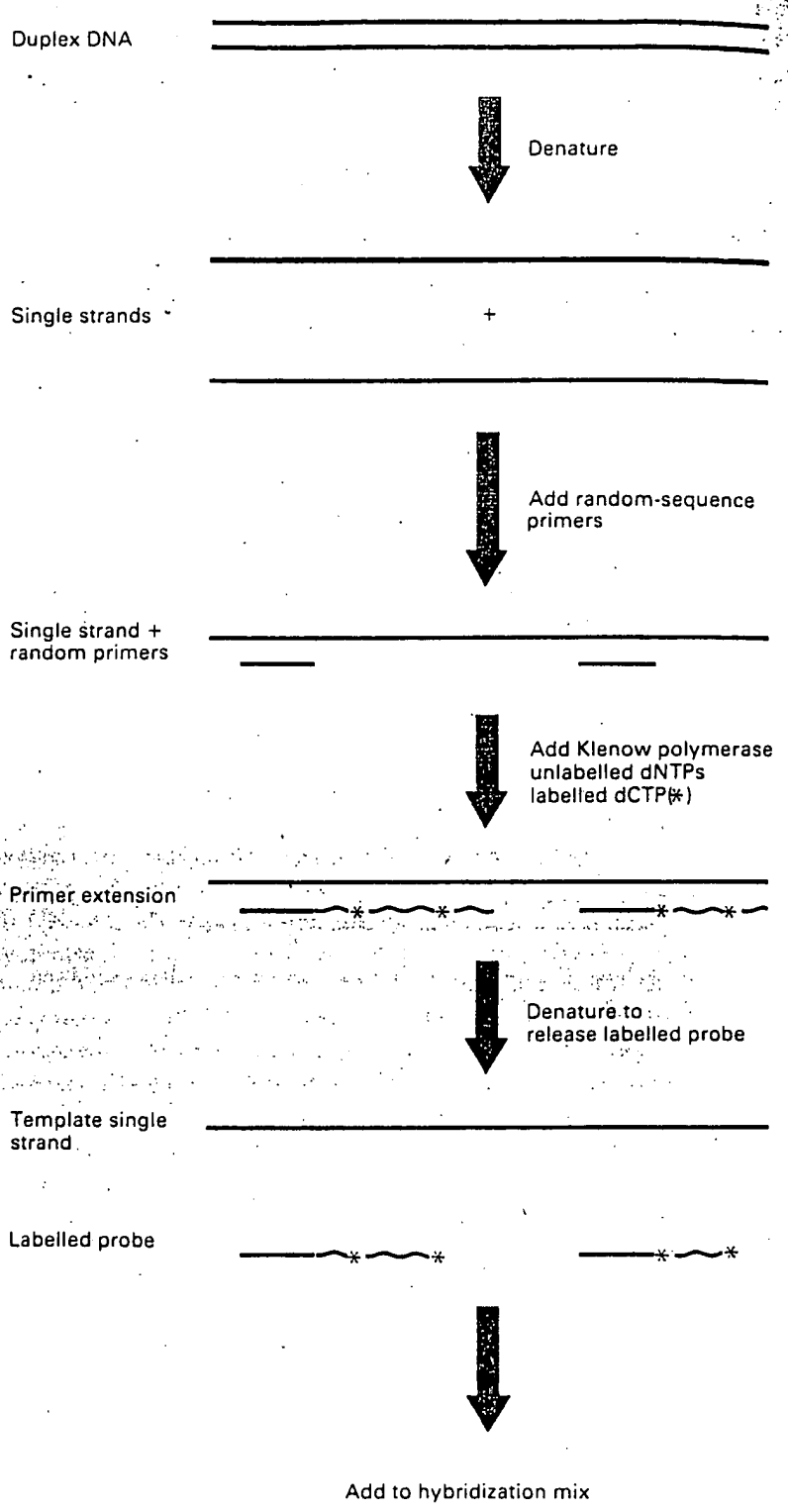


Fig. 15.2 The random primer method for preparing labelled DNA.

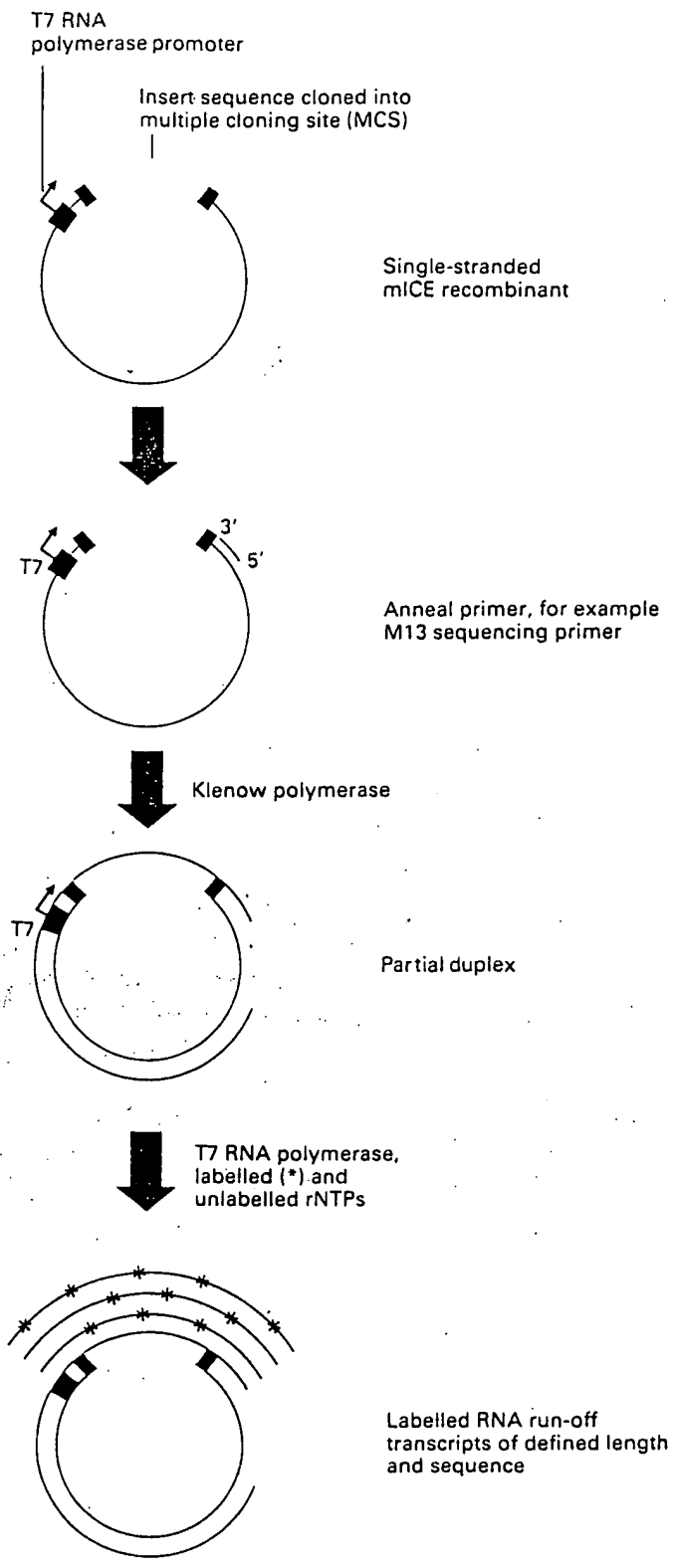


Fig. 15.4 Preparation of probes using a single-stranded mICE vector.

target and the probe nucleic acid are free to move, thus maximizing the chance that complementary sequences will align and bind. Consequently solution hybridizations go to completion five- to 10-fold faster than those on solid supports (Bryan *et al.* 1986). This can be particularly important in many diagnostic microbiology applications where the concentration of the target sequence is very low and speed is essential.

At the end of the hybridization step it is essential to separate duplexes from unbound probe. If one of the two sequences in the hybridization reaction has been immobilized this separation step is achieved by a simple washing procedure. This explains the popularity of filter hybridization, of which there have been numerous examples in previous chapters.

A variation of the filter hybridization reaction is to attach the probe to the bottom of a microtitre plate well or to a tube (Polsky-Cynkin *et al.* 1985). This facilitates the washing step, reduces the total volume of the hybridization, and facilitates the automated reading of results if a colorimetric detection system is used. A clever utilization of this format is the sandwich hybridization reaction (Ranki *et al.* 1983, Palva & Ranki 1985). Here one probe is attached to the solid support and serves to capture homologous nucleic acids. A second DNA probe, which recognizes a contiguous sequence carries the reporter molecule (Fig. 15.12). Although the sandwich format is far less dependent upon the sample composition than direct blotting methods, it is a relatively slow process. Also, the capture is inefficient, since after denaturing the target DNA, the rate of reassociation of the target with itself in solution is considerably greater than its rate of association with the solid-phase probe (Syvanen *et al.* 1986). For the latter reason RNA probes are preferable to DNA probes.

If solution hybridization has been used, removal of unbound probe is not easy. One method is to digest the single-stranded nucleic acid that remains after hybridization with an appropriate nuclease. Although attractive in principle, in practice efficient digestion and separation are difficult to achieve reliably, particularly with crude samples. An alternative method is to separate duplexes from single-stranded by means of differential binding to hydroxyapatite or antibodies specific for double-stranded nucleic acids. A different approach has been adopted by Gingeras *et al.* (1987) who carried out sandwich hybridization in solution. The target DNA is hybridized in solution to a labelled probe and to an unlabelled capture probe which is immobilized by covalent attachment at its 5′ end to a solid support. If the solid support is in the form of beads, subsequent

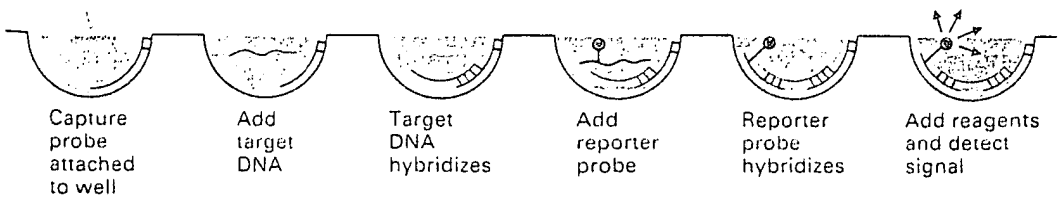
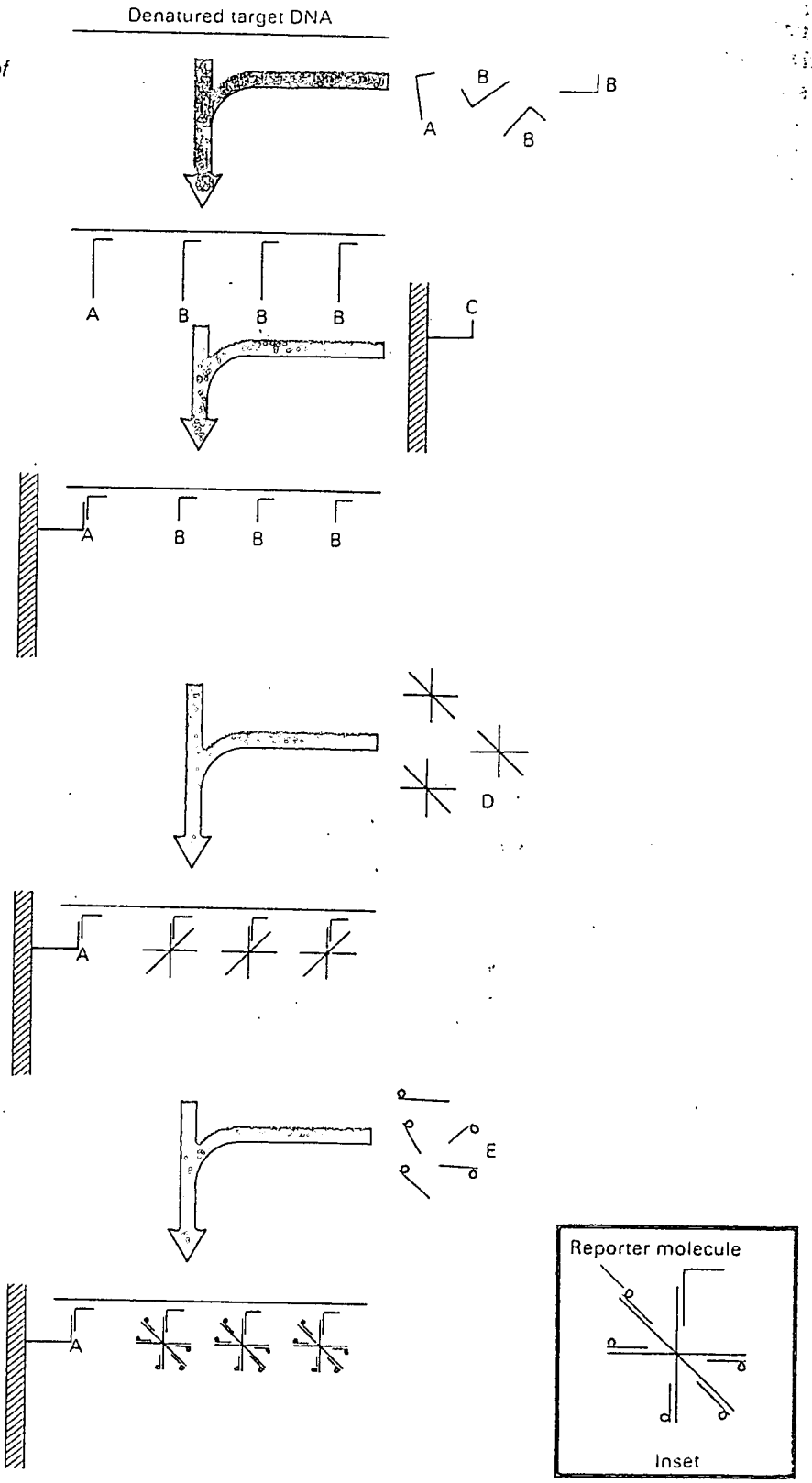


Fig. 15.12 The sandwich hybridization method as carried out in a microtitre tray. The different wells of the microtitre tray show different stages in the procedure.


Denatured target DNA
A
B
B
B
A
B
B
B
C
A
B
B
B
D
A
E
A
Reporter molecule
Inset